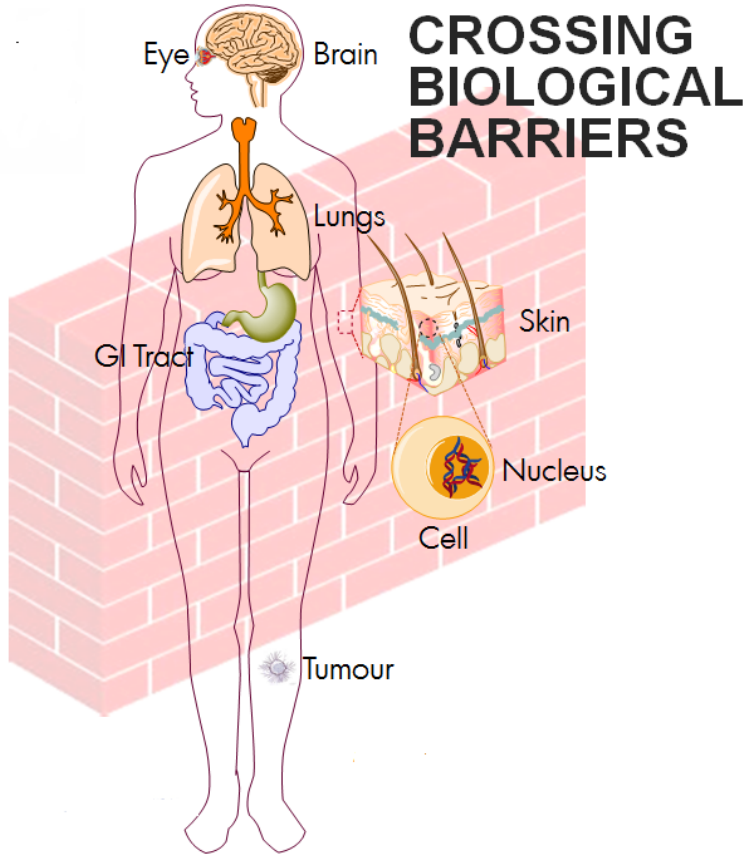
Self-assembled Peptides as Drug Delivery Molecules for transport across the Biological Barriers

OVERCOMING BIOLOGICAL BARRIERS: PEPTIDE NANOTUBES FOR DRUG DELIVERY

Biological barriers

- Biological barriers inhibit drug delivery throughout the body
- For efficient drug delivery, drugs must be able to reach their target site of action
- The blood brain barrier is very problematic for drug delivery
- Large gap in adequate care for patients for particular diseases: brain tumors, Parkinson's, Alzheimer's



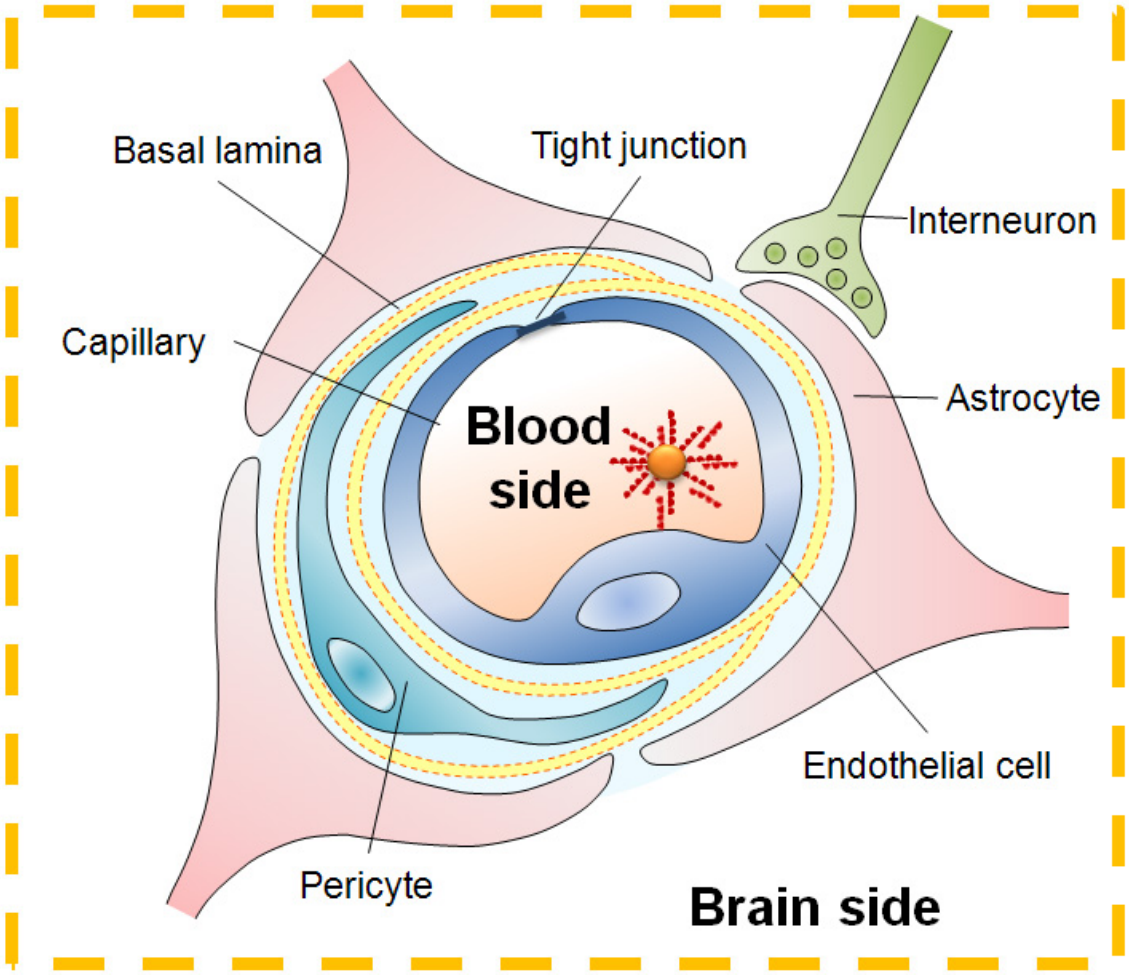
CRS. 2015. Controlled release society. [ONLINE] Available at: http://www.crsaustralia.org/?page_id=971. [Accessed 16 April 2017].

The blood brain barrier

For Transmembrane diffusion of small molecules: <400 Daltons and high lipid solubility

Approaches to bypassing this barrier:

- Disruption of the blood brain barrier tight junctions (detergents or ultrasound)
- Receptor mediated transcytosis – engage cell-surface receptors over expressed by brain cells.
- Adsorptive endocytosis of nanoparticles

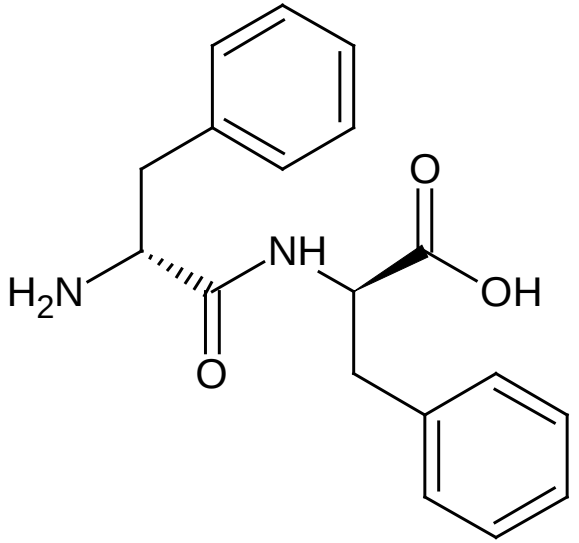


Jonathan C.H. Choi . 2016. Jonathan C.H. Choi Research Group. [ONLINE] Available at: http://www.bme.cuhk.edu.hk/jchchoi/r_SHIAE14-16.php. [Accessed 16 April 2017].

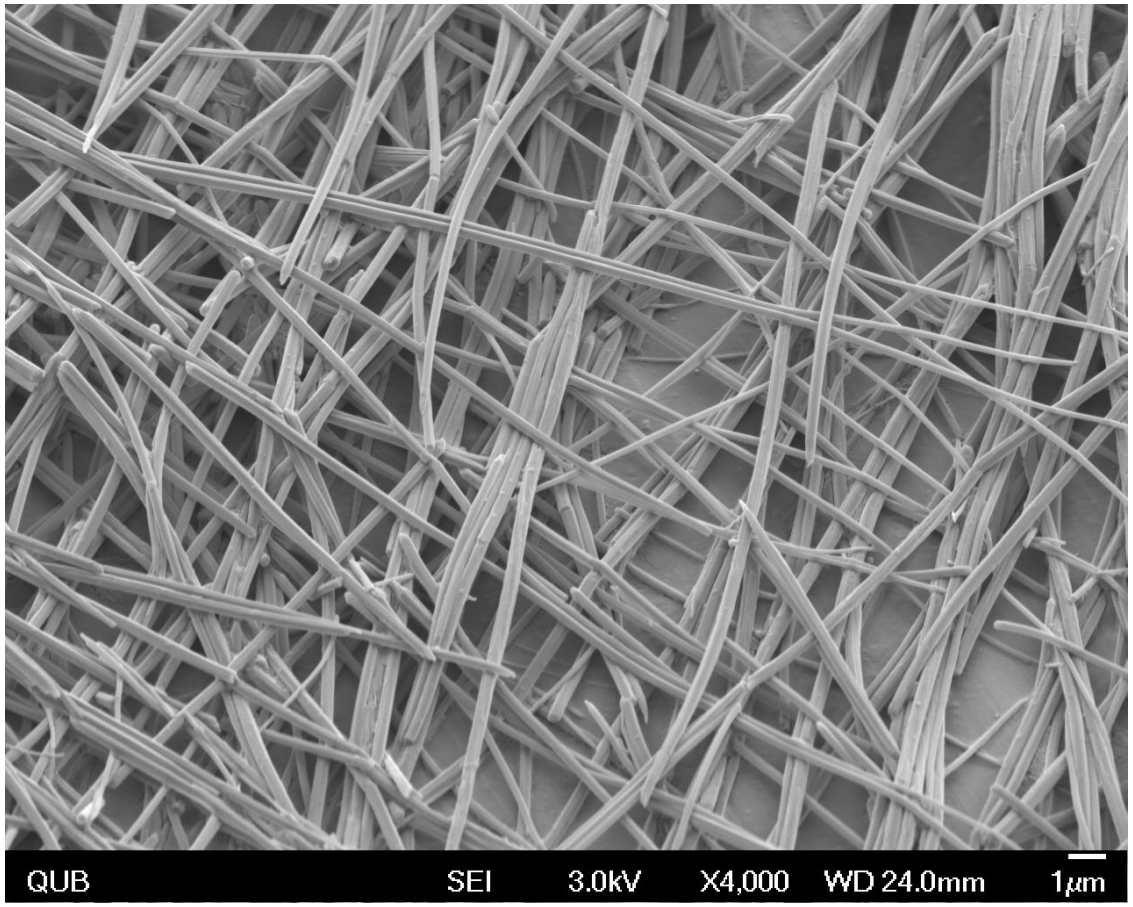
Peptide structure

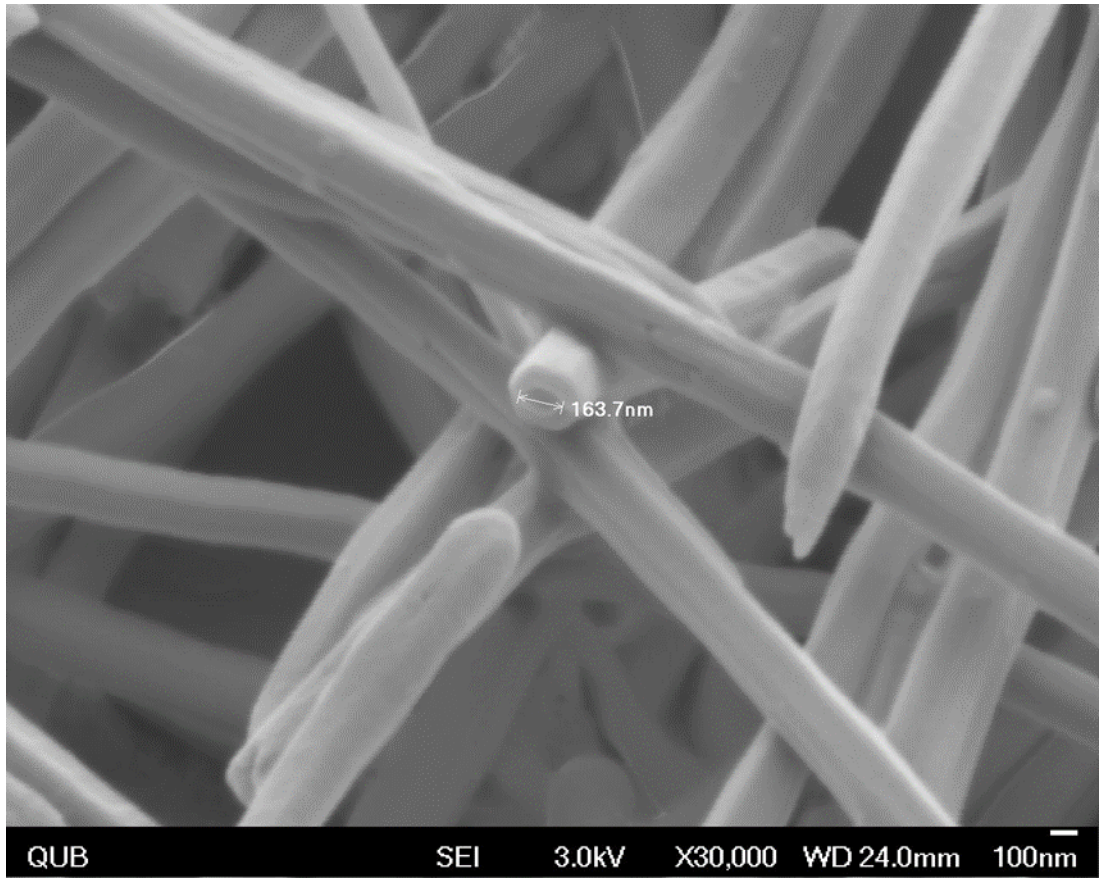
- Ultra short peptide structure consisting of two amino acids
- Phenylalanine-Phenylalanine
- Molecular weight of 312.36 Daltons
- Relatively easy manufacture process due to the short length – peptides become increasingly difficult to manufacture at high purities with increasing chain length

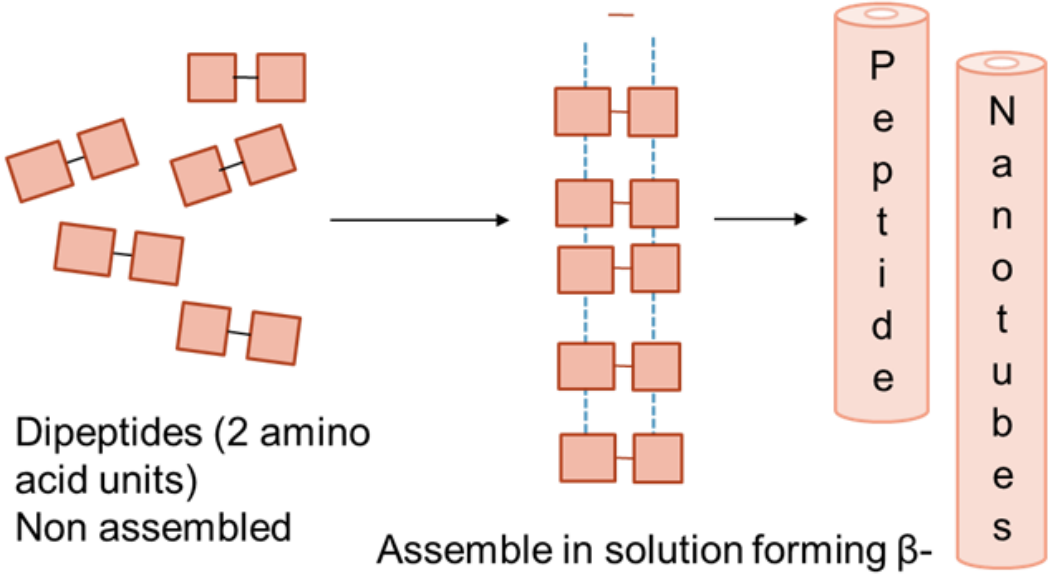
NH_2-($_D$)Phe-($_D$)Phe-COOH



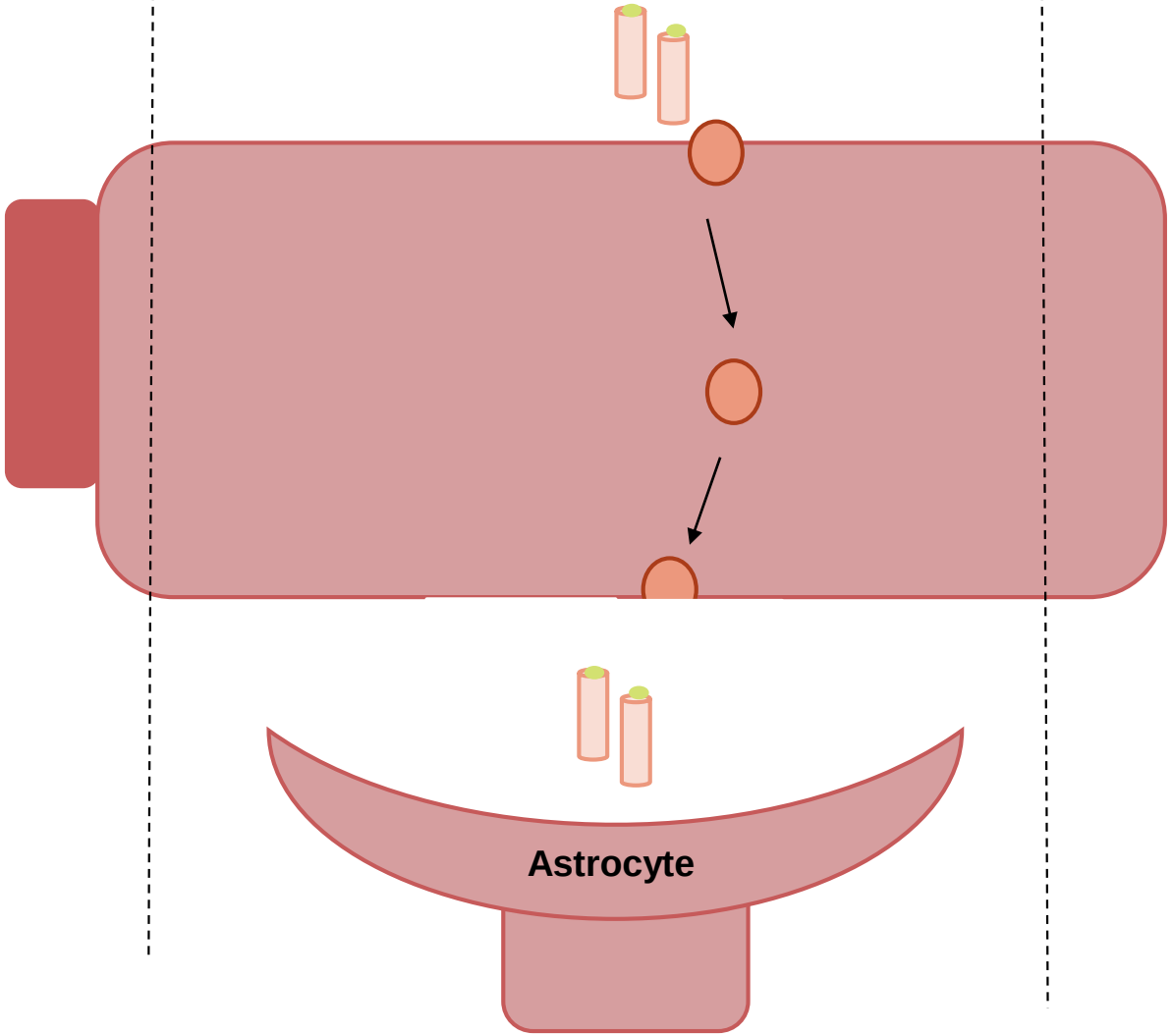
Self assembled peptide nanotubes



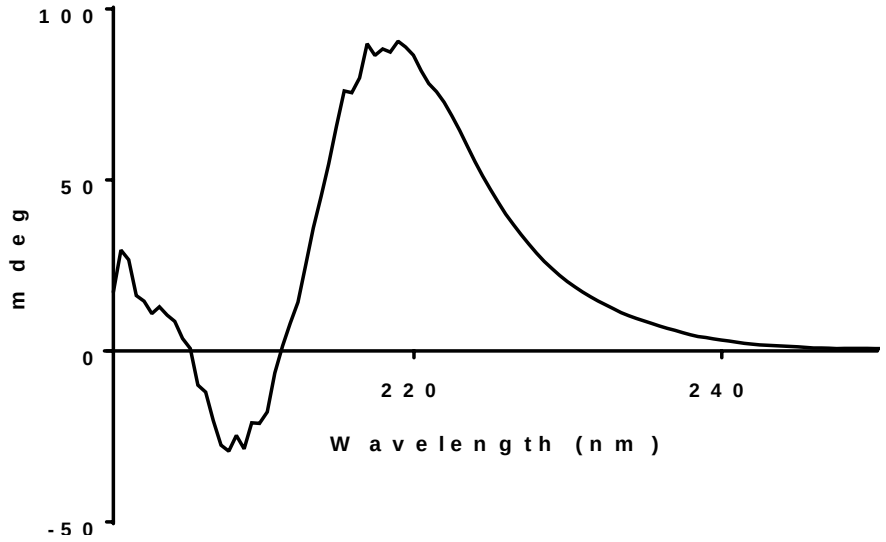




B: Adsorptive endocytosis (Previously demonstrated for nanotube structures)



Characterising the assembled secondary structure



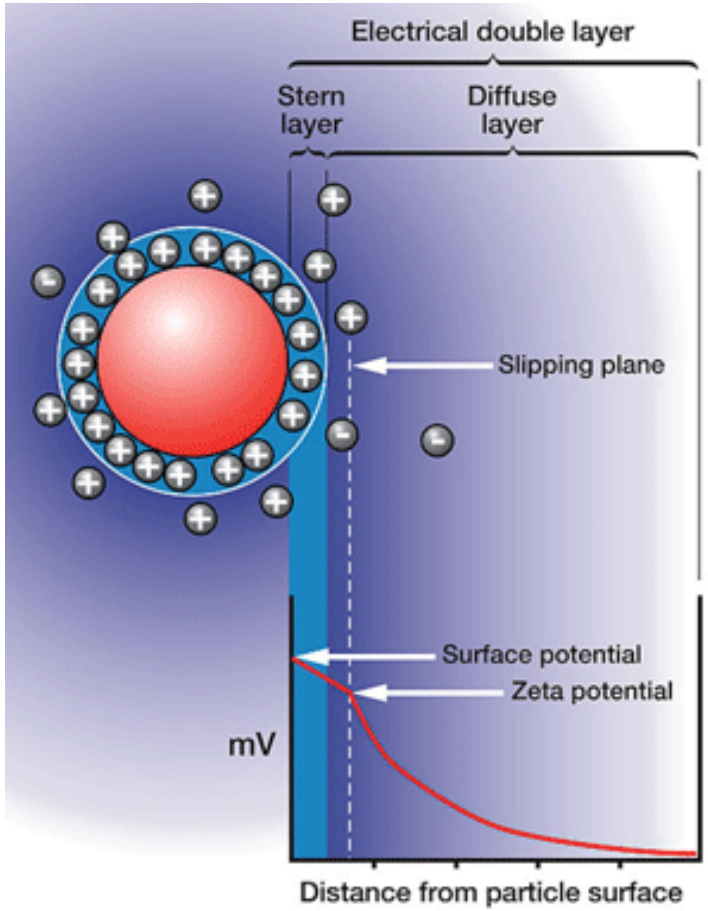
Circular dichroism spectra for assembled peptide nantotube structures:

- Large positive peak ~ 217 nm
- Characteristic of β-sheet assembly in peptides
- Useful to confirm presence of peptide nanotubes in absence of environmental microscope

Measuring zeta potential

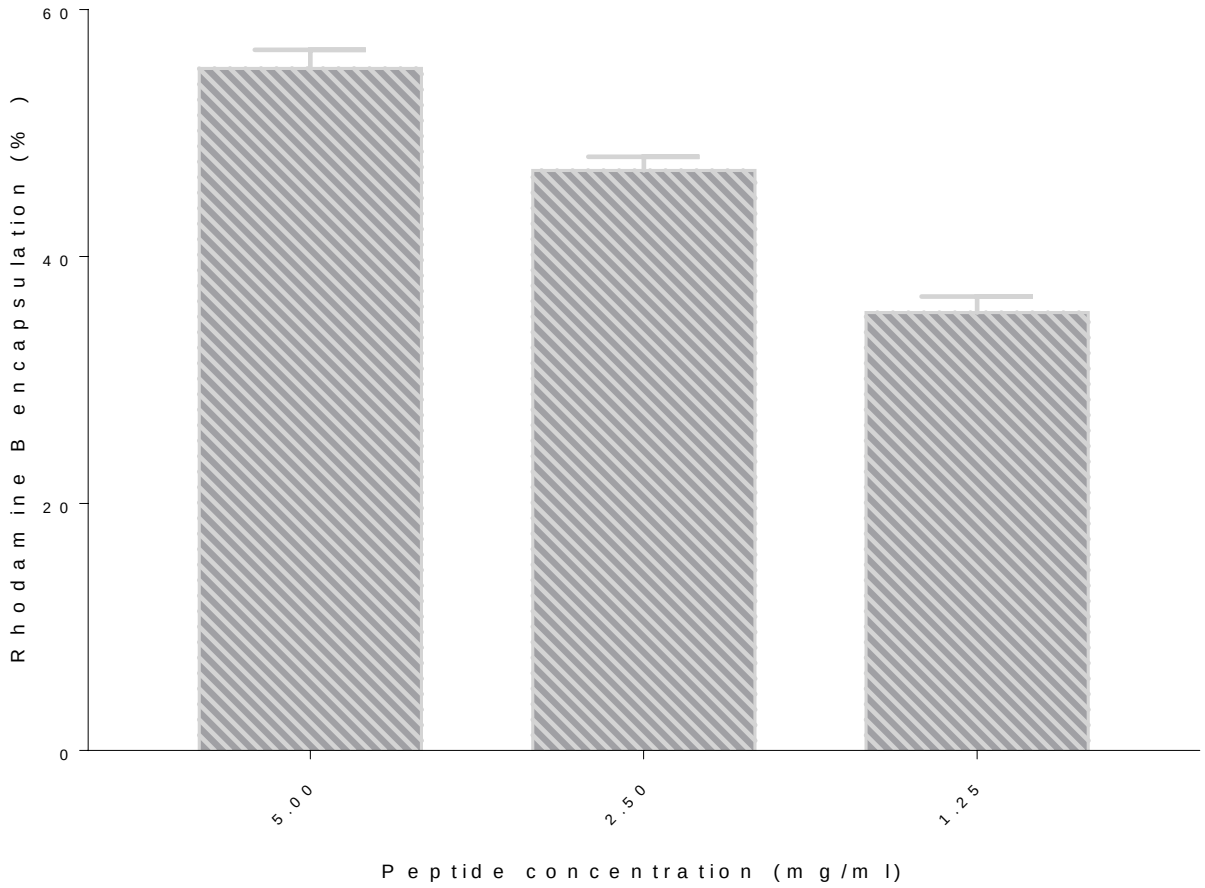
- Particle charge is a key consideration when considering formulation of any nanoparticle
- Unfavourable charge pairing will lead to the repelling of the drug cargo from the nanoparticle
- Extremes of either positive or negative charge unfavourable for delivering across cell layers because it can lead to entrapment

pH (n=3)	Mean Zeta Potential (mV)
7.4	-21.10(±0.49)
5.5	-13.57(±2.37)



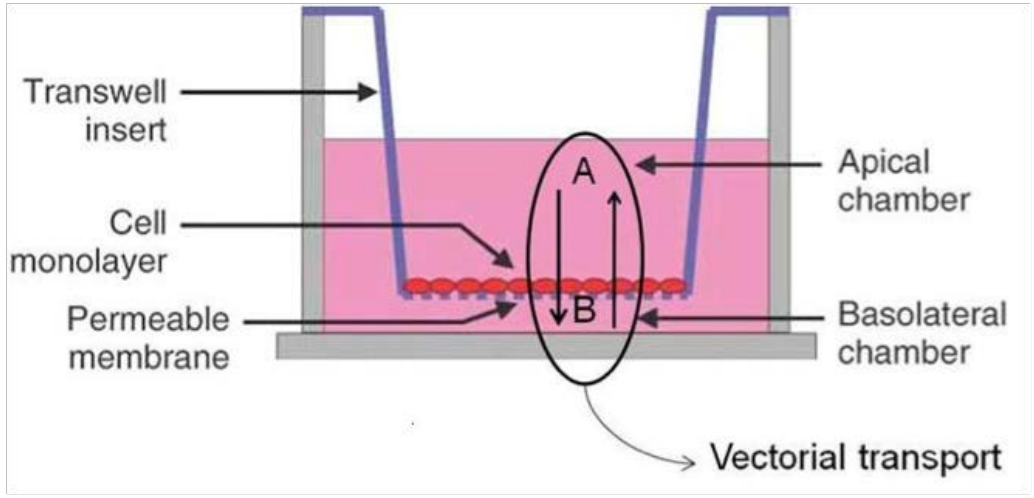
Water World. 2016. ONLINE ZETA POTENTIAL MEASUREMENT PROVIDES WATER TREATMENT CONTROL, COST REDUCTION. [ONLINE] Available at: http://www.waterworld.com/articles/print/volume-30/issue-10/features/automation-technology/online-zeta-potential-measurement-provides-water-treatment-control-cost-reduction.html. [Accessed 16 April 2017].

Encapsulation of Rhodamine B



- Instead of beginning encapsulation and permeation studies with a drug, a fluorescent molecule is used for ease of screening
- Rhodamine B is chosen as a tracer molecule due to the previous zeta potential data, rhodamine should be positively charged at pH during the assembly process
- Rhodamine B is added while the peptide nanotubes are self assembling and are spontaneously incorporated into the tubes
- A maximum of 56 % encapsulation efficiency was observed

In-vitro blood brain barrier model

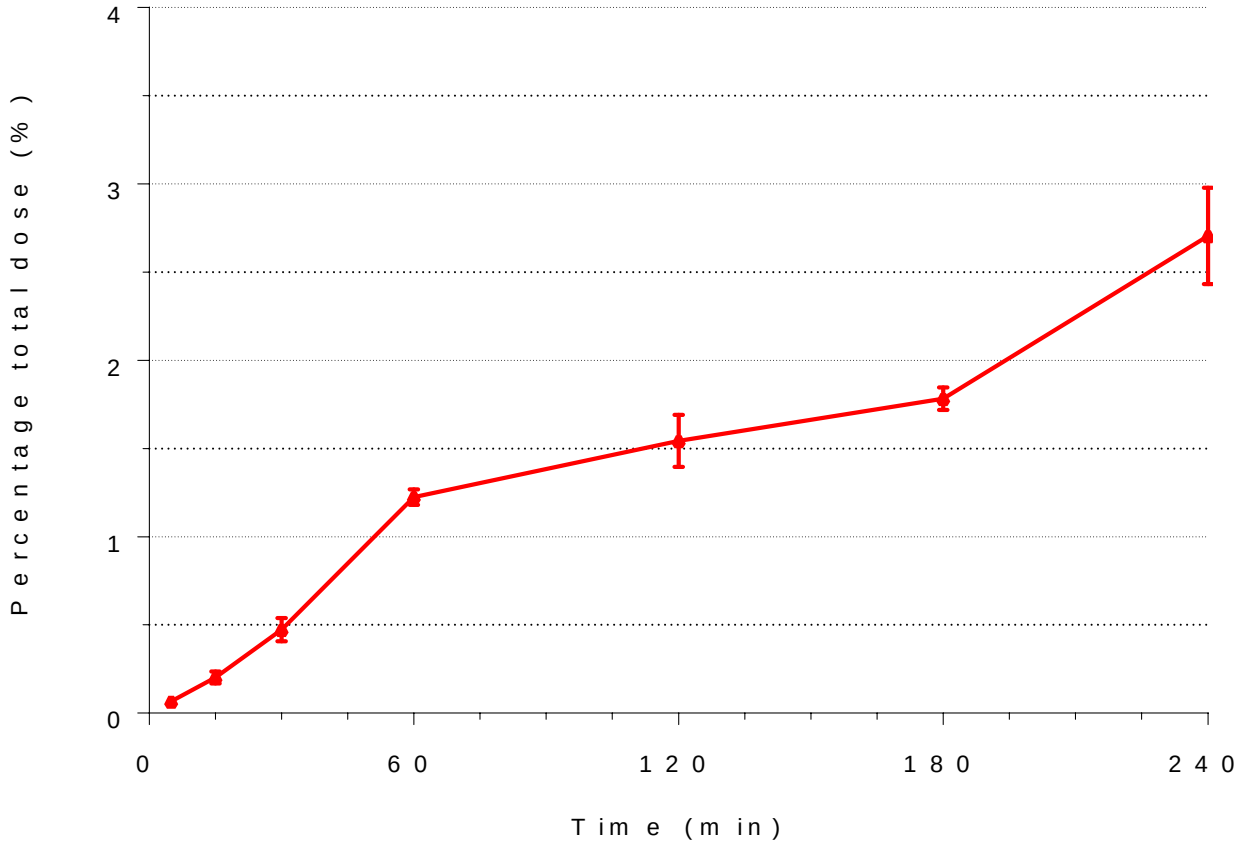


- Transwell inserts used to grow a brain endothelial cell layer on
- Semi-porous membrane containing defined pore size (0.4 micron)
- Cell layer grown on insert membrane – creates two separate chambers separated by a brain endothelial cell layer (hCMEC/D3 Cell)
- Nanotube suspensions loaded with a fluorescent marker can be loaded into top chamber
- Validate using TEER

Brunswick laboratories. 2016. Caco-2 Permeability Screening Assay (unidirectional/bidirectional). [ONLINE] Available at: https://brunswicklabs.com/capabilities/clinical-study/adme-tox-studies/caco-2-permeability-screening/. [Accessed 16 April 2017].

Permeation across model

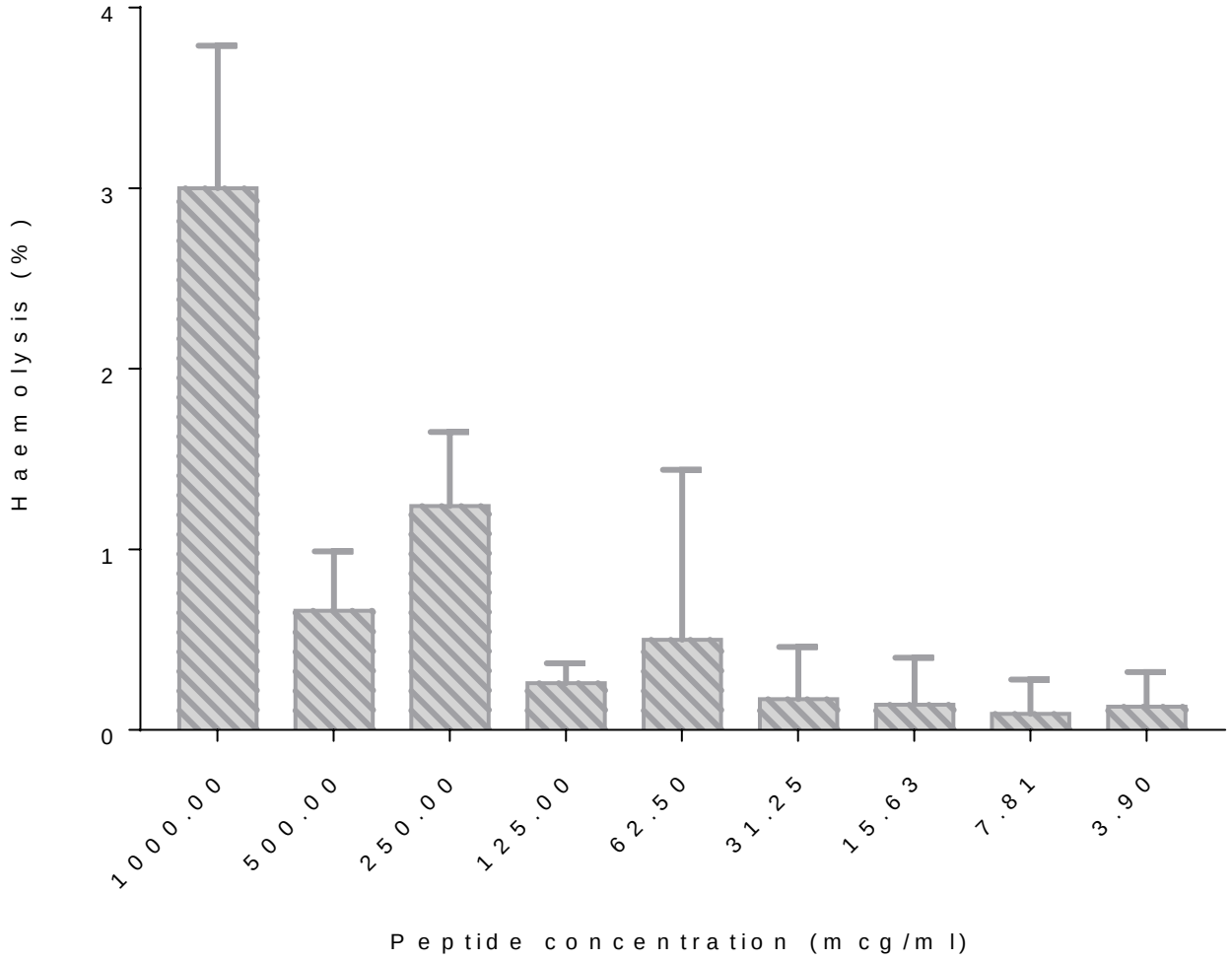
- At time zero peptide nanotube suspension loaded with rhodamine b is added to the top chamber of the transwell insert
- The bottom chamber is sampled over time and the fluorescence intensity of rhodamine b at 562/583 nm excitation/emission wavelengths is recorded
- Peptide nanotubes transport rhodamine over the cell monolayer and then begin to de-assemble once they have reached the bottom compartment and are no longer in a peptide monomer saturated solution
- After 4 hours 2.4% of the total dose had crossed, which is reasonable considering the short time point

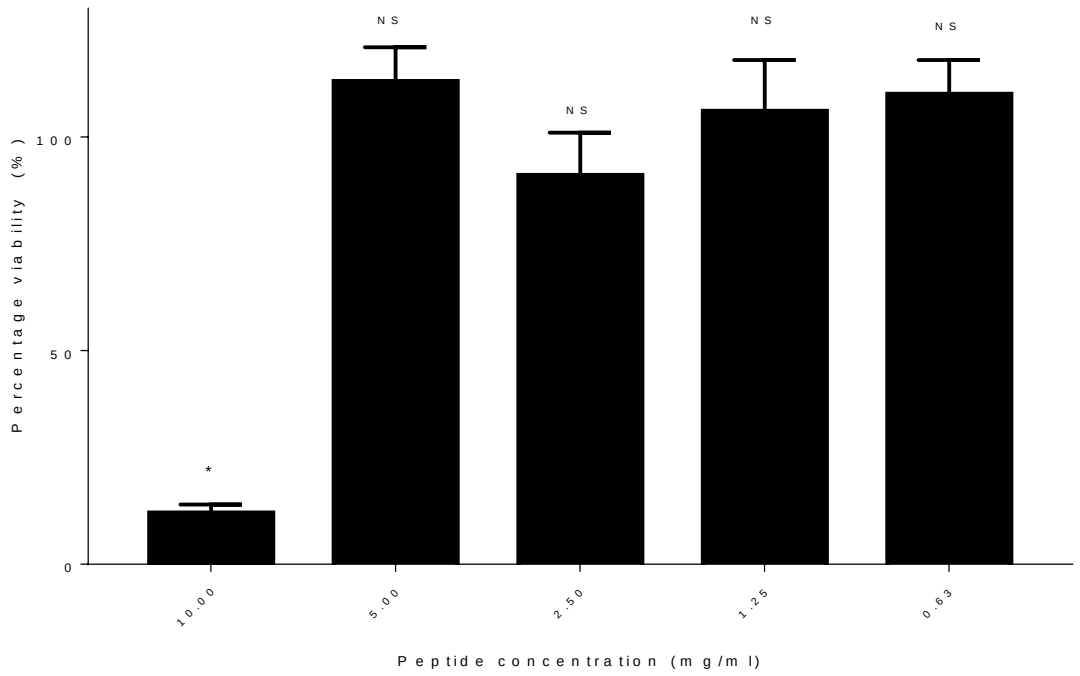


Biocompatibility studies

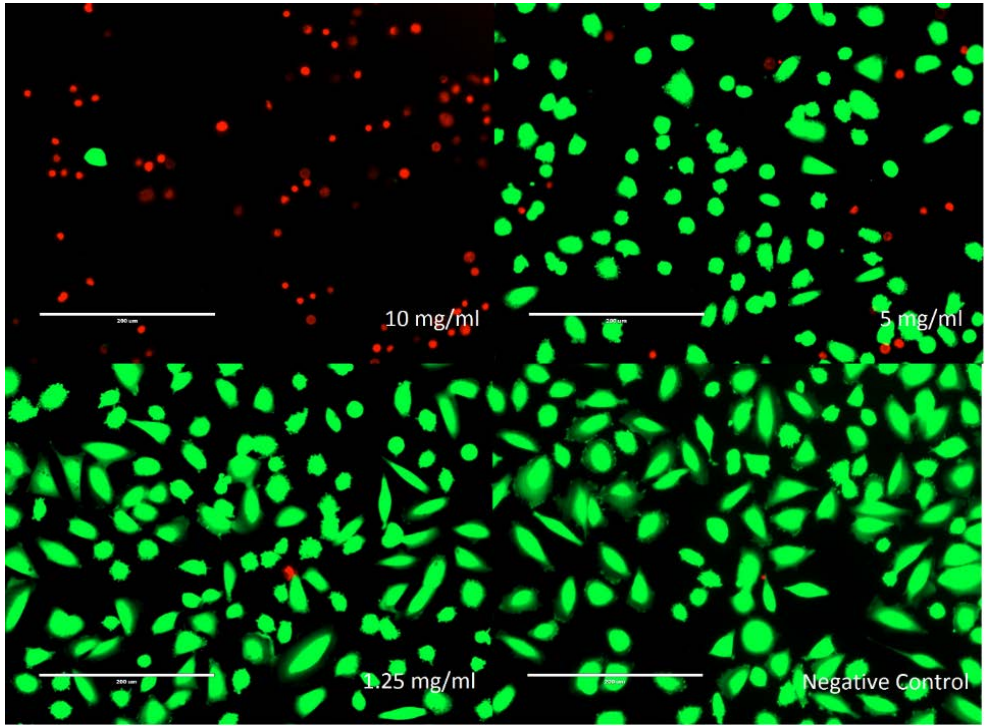
Haemolysis of equine erythrocytes:

- Peptide nanotubes are incubated with horse red blood cells
- The percentage of red blood cells lysed can be calculated by measuring absorbance
- Gives an indicator of how compatible the treatments would be as an injectable





Percentage of remaining viable NCTC 929 cells following 6-hour treatment with a range of peptide nanotube suspension concentrations using a MTS assay. Key: black column:. NS: no significant difference (P≥0.05), *: P<0.01 significant difference between percentage viability of peptide nanotube treated cells and negative control.



LIVE/DEAD® stain of NCTC 929 cells following 6-hour treatment with peptide nanotube suspension. Live cells are stained green and dead cells are stained red. Scale bar represents 200 µm.

Conclusions

- Phenylalanine-phenylalanine peptides can self assemble into nanotube structures which can encapsulate fluorescent markers
- Preliminary studies have shown they have high biocompatibility and can permeate an *in vitro* blood brain barrier model

Thank you